New England Biolabs
Product Specification

Product Name: Mismatch Endonuclease I
Catalog #: M0678S
Concentration: 80,000 units/ml
Unit Definition: One unit is defined as the amount of enzyme required to cleave ≥50% of 0.2 pmol of a fluorescently labeled 60mer oligonucleotide duplex containing a single T-T mismatch in 30 minutes at 37°C in a total reaction volume of 20 µl in 1X NEBuffer r2.1.
Shelf Life: 24 months
Storage Temp: -20°C
Storage Conditions: 500 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, (pH 7.4 @ 25°C)
Specification Version: PS-M0678S v1.0
Effective Date: 02 Jul 2021

Assay Name/Specification (minimum release criteria)

**DNase Activity (Labeled Oligo, 3’ extension)** - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3’ extension and a minimum of 5 µl of Mismatch Endonuclease I incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

**Double Stranded DNase Activity (Labeled Oligo)** - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 5 µl of Mismatch Endonuclease I incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda-HindIII DNA and a minimum of 400 units of Mismatch Endonuclease I incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Protein Purity Assay (SDS-PAGE)** - Mismatch Endonuclease I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**qPCR DNA Contamination (E. coli Genomic)** - A minimum of 80 units of Mismatch Endonuclease I is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.
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<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tr>
<td><strong>RNase Activity (Extended Digestion)</strong> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 80 units of Mismatch Endonuclease I is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
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<td><strong>Single Stranded DNase Activity (FAM-Labeled Oligo)</strong> - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 5 µl of Mismatch Endonuclease I incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</td>
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Derek Robinson
Director, Quality Control

Date 02 Jul 2021